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Autoreactive T Cells Mediate NK Cell Degeneration in Autoimmune Disease

Ruolan Liu,* Luc Van Kaer,† Antonio La Cava,‡ Mary Price,* Denise I. Campagnolo,* Mary Collins,§ Deborah A. Young,§ Timothy L. Vollmer,* and Fu-Dong Shi2*#

Emerging evidence indicates that NK cells play an important and complex role in autoimmune disease. Humans with autoimmune diseases often have reduced NK cell numbers and compromised NK cell functions. Mechanisms underlying this NK cell degeneration and its biological significance are not known. In this study we show that, in an experimental model of human autoimmune myasthenia gravis induced by a self-Ag, the acetylcholine receptor, NK cells undergo proliferation during the initiation of autoimmunity, followed by significant degeneration associated with the establishment of the autoreactive T cell response. We show that NK cell degeneration was mediated by IL-21 derived from autoreactive CD4+ T cells, and that acetylcholine receptor-immunized IL-21R-deficient mice, with competent NK cells, developed exacerbated autoimmunity. Thus, NK cell degeneration may serve as a means evolved by the immune system to control excessive autoimmunity. The Journal of Immunology, 2006, 176: 5247–5254.

Natural killer cells are large granular lymphocytes of the innate immune system and respond rapidly to a variety of insults via cytokine secretion and cytolytic activity (1–4). Recently, there has been growing insight into the biological function of NK cells, particularly with regard to their role in the regulation of autoimmunity in animal models and in humans (5–7). Some studies suggest that NK cells prevent or curb autoimmune responses (8–11). Other results indicate that NK cells have a permissive role in autoimmunity, perhaps due to their ability to provide an early source of cytokines and to activate APCs leading to the development of pathogenic Th1 responses (12–16). Clearly, these studies imply that NK cells can regulate inflammation and loss of self-tolerance at multiple steps and, therefore, suggest that NK cells play distinct roles during different forms and at different stages of autoimmunity.

Although factors that account for the apparently opposing effects of NK cells in autoimmune disease remain to be identified, one finding emerges consistently: NK cell numbers and functions decline during the progression of most autoimmune diseases of humans (17–19). Mechanisms underlying NK cell degeneration in autoimmune disease remain elusive. Environmental triggering coupled with genetic susceptibility is necessary to precipitate overt autoimmune disease. Although infections are frequently associated with relapses of autoimmune disease (20), they may not be required to maintain autoimmunity, once initiated. Additionally, NK cells in environments of ongoing autoimmunity may be confronted with excess quantities of cytokines derived from autoreactive T and B cells. The temporal concordance of decreased NK cell responses with the emergence of Ag-specific T cells makes it likely that T cells and T cell-derived factors influence the fate of NK cells. Therefore, we hypothesized that (1) NK cell degeneration stems from a lack of sustained NK cell stimuli during the progression of autoimmune disease; or alternatively, (2) NK cell degeneration is mediated by autoreactive T and/or B cells.

In this study, we demonstrate that NK cell degeneration in experimental autoimmune myasthenia gravis (EAMG) does not result from a lack of sustained stimuli but is, instead, mediated by IL-21 derived from autoreactive CD4+ T cells. Acetylcholine receptor (AChR)-immunized IL-21R-deficient mice, with competent NK cells, developed exacerbated autoimmunity. Our results provide a mechanistic explanation for the long-standing puzzle of NK cell degeneration during the progression of autoimmune diseases and suggest that NK cell degeneration significantly influences the course of autoimmunity.

Materials and Methods

Mice

C57BL/6 (B6) mice were purchased from Charles River Laboratories or The Jackson Laboratory. RAG1−/− (21). B cell-deficient μMT, CD4−/−, CD8−/−, and IL-12−/− mice were purchased from The Jackson Laboratory. IL-18−/− mice (22) were provided by Drs. K. Takeda and S. Akira (Japan Science and Technology Agency, Osaka, Japan). IL-15−/− mice (23) were provided by Dr. J. J. Peschon (Immunex; Seattle, WA). IL-21R−/− mice (24) and CD1d−/− mice (25) have been described. All mutant mice were backcrossed 10× to a B6 background.

Induction of EAMG

AChR was purified from the electric organs of Torpedo californica (Pacific Biomarine) by affinity chromatography on a α-cobratoxin-agarose resin (Sigma-Aldrich) (26). An inoculum of 20 μg of AChR in CFA in a total volume of 100 μl was used to immunize mice s.c. along the shoulders and back. Mice were boosted twice at days 30 and 60 with 20 μg of AChR in CFA or IFA s.c. at four sites on the shoulders and thighs. Clinical manifestations of EAMG were graded between 0 and 3 with standard criteria (14). Cumulative disease scores were calculated by adding disease scores weekly from the onset to termination of the experiment.
NK cell isolation

NK cells (NK1.1^+ CD3^-) from pooled splenocytes were enriched with anti-mouse CD5b/pan-NK cell marker (BD Biosciences) or with magnetic beads for CD5 (Miltenyi Biotech). After removal of CD5^+ cells, NK cells were sorted from the remaining cells by FACSAria or FACSStar using Diva software (BD Biosciences) (9, 10). Purity of NK cells obtained by this approach reached 98%.

NK: T cell coculture

AChR-reactive CD4^+ T cells were selected using a split-well assay (27). A total of 1–3 × 10^5 NK cells and AChR-reactive CD4^+ T cells/well were cultured together in 48-well plates for 18 h. In some experiments, NK cells and T cells were cultured with a permeable membrane (Transwell) of 6.5-mm diameter and 5-μm pore size (Costar).

FIGURE 1. NK cell degeneration during the progression of autoimmunity. Mice were immunized with AChR/CFA at 3 monthly intervals to induce EAMG. Control mice were immunized with CFA alone at the same frequency. (a) Representative plots from three separate experiments (n = 4 mice/group) show the CFSE dilution of gated NK1.1^- CD3^- cells from splenocytes purified from mice at day 3 after immunization with AChR/CFA. Numbers (b) and percentages (c and d) of NK cells among splenocytes of B6 mice immunized with CFA alone (c) (n = 12 for each time point) or B6 mice immunized with AChR in CFA (d) (n = 6 for each time point) at different time points p.i. e, NK cell-mediated cytotoxicity of splenocytes from naive B6 mice (n = 12), B6 mice immunized with CFA alone (n = 12), or B6 mice immunized with AChR in CFA (n = 4) at days 1, 3, 7, 15, 35, and 75 p.i. Spleen cells were incubated with ^51Cr-labeled YAC-1 target cells at an E:T ratio of 100:1. After 4 h of culture, supernatants were counted for ^51Cr release in a gamma counter. f, Mean numbers (±SD) of IFN-γ spot-forming colonies of NK cells measured by ELISPOT assay. NK cells from spleen cells of mice indicated in e were enriched and sorted by flow cytometry and cultured for 48 h without Ag stimulation. g, T cell proliferation in response to AChR and α146-162 peptide (data not shown) was measured by using [3H] incorporation assay for the groups of mice indicated in a. h, Mean numbers of anti-AChR Ab-producing B cells from groups of mice indicated in a were measured by ELISPOT assay. Data shown represent one of three experiments. Similar results were seen in draining lymph nodes (data not shown). Statistical comparisons were performed between AChR/CFA and CFA groups, or between time points within a group. *, p < 0.05; **, p < 0.01.
were analyzed by FACS. Mean numbers (±SD) of IFN-γ spot-forming colonies were measured by ELISPOT. Results represent one of two experiments. Comparisons were made between day 1 p.i. and day 61 p.i. within the same group of mice.

We began our investigation by immune-phenotyping and functional characterization of NK cells during the course of EAMG. In mice immunized with AChR/CFA, splenic NK cells appeared to undergo rapid proliferation from day 1 to 3 postimmunization (p.i.), as evidenced by dilution of CFSE on gated NK cells (Fig. 1a), followed by a numerical decline after day 7 p.i. By day 75 p.i., the percentage and absolute number of NK cells (NK1.1−CD3+CD24+) was approximately one-fourth of that in the induction phase of EAMG (Fig. 1, b and d). The dynamic changes in the NK cell compartment during the initiation of EAMG appeared to be the result of NK cell proliferation in situ rather than recruitment, because modest NK cell expansion was also observed in lymph nodes, peripheral blood, and thymus (data not shown). In contrast, there was a moderate increase in the percentage of NK cell in mice injected with CFA at the same frequency, which was maintained throughout the 100-day observation period (Fig. 1, b and c). NK cells from mice treated with AChR/CFA rapidly acquired the ability to lyse YAC-1 target cells (Fig. 1e) and to spontaneously produce IFN-γ (Fig. 1f) at days 3 and 7 p.i. However, from day 15 p.i., NK cell activity started to decline. This deficit in NK cell function was also observed when purified NK cells were activated with LPS (1 μg/ml) (numbers of IFN-γ spot-forming cells in EAMG mice were 47 ± 11 vs 13 ± 4 on day 3 and 75 p.i.). In contrast, mice immunized with CFA alone maintained NK cell functions at a magnitude similar to that preceding their initial priming (Fig. 1, e and f).

We also examined the kinetics of T cell and autoantibody responses to AChR and its dominant epitope α146–162 during the course of EAMG. Vigorous T cell proliferation and anti-AChR-producing B cells in response to AChR and α146–162 emerged around day 6 and 8 p.i., respectively (Fig. 1, g and h, and data not shown), just before the decline in NK cell functions. We observed a similar pattern of NK cell degeneration during progression of the autoimmune response to myelin Ags, including myelin basic protein and myelin oligodendrocyte glycoprotein, whereas the number and functions of NK cells were maintained in mice immunized with CFA and OVA (F.-U. Shi, unpublished observation). These results suggest that 1) NK cells undergo significant degeneration during the transition from EAMG initiation to disease progression; 2) the observed decline in NK cell functions is not caused by the lack of continuous stimuli; and 3) this transition in NK cell function is associated with the emergence of autoreactive T and B cell responses.

Table I. Preservation of NK cell functions in mice devoid of CD4+ T cells

<table>
<thead>
<tr>
<th>Groups (mice)</th>
<th>No. of Mice</th>
<th>Treatment (%)</th>
<th>Percentage of NK Cells (%) SD Day 1 p.i.</th>
<th>Percentage of NK Cells (%) SD Day 61 p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. RAG1−/−</td>
<td>5</td>
<td>CFA</td>
<td>23.4 ± 6.3 (105)</td>
<td>20.9 ± 8.1 (105)</td>
</tr>
<tr>
<td>2. RAG1−/−</td>
<td>4</td>
<td>AChR/CFA</td>
<td>27.8 ± 12.1 (105)</td>
<td>29.5 ± 6.6 (105)</td>
</tr>
<tr>
<td>3. C57BL6</td>
<td>4</td>
<td>AChR/CFA</td>
<td>2.7 ± 0.5 (105)</td>
<td>0.5 ± 0.5 (105)</td>
</tr>
<tr>
<td>4. µMt</td>
<td>6</td>
<td>AChR/CFA</td>
<td>3.2 ± 0.7 (105)</td>
<td>1.3 ± 0.9 (105)</td>
</tr>
<tr>
<td>5. C57BL6</td>
<td>5</td>
<td>AChR/CFA</td>
<td>2.4 ± 0.3 (105)</td>
<td>0.7 ± 0.1 (105)</td>
</tr>
<tr>
<td>6. CD4−/−</td>
<td>4</td>
<td>AChR/CFA</td>
<td>3.4 ± 0.5 (105)</td>
<td>3.6 ± 0.6 (105)</td>
</tr>
<tr>
<td>7. C57BL6</td>
<td>5</td>
<td>AChR/CFA</td>
<td>2.3 ± 0.4 (105)</td>
<td>0.8 ± 0.2 (105)</td>
</tr>
<tr>
<td>8. CD8−/−</td>
<td>4</td>
<td>AChR/CFA</td>
<td>1.0 ± 0.4 (105)</td>
<td>1.0 ± 0.4 (105)</td>
</tr>
<tr>
<td>9. C57BL6</td>
<td>4</td>
<td>AChR/CFA</td>
<td>2.4 ± 0.2 (105)</td>
<td>0.5 ± 0.2 (105)</td>
</tr>
<tr>
<td>10. CD14−/−</td>
<td>8</td>
<td>AChR/CFA</td>
<td>2.1 ± 0.5 (105)</td>
<td>0.7 ± 0.2 (105)</td>
</tr>
</tbody>
</table>

*Groups of mice were immunized with AChR/CFA or CFA alone at 3 monthly intervals. Percentages of NK cells in splenocytes at day 1 and 61 after primary immunization were analyzed by FACS. Mean numbers (±SD) of IFN-γ spot-forming colonies were measured by ELISPOT. Results represent one of two experiments. Comparisons were made between day 1 p.i. and day 61 p.i. within the same group of mice. *p < 0.05; **p < 0.01.

NK cell depletion

Mice were given injections i.p. with 250 μg of anti-NK1.1 mAb (PK136 clone) for initial depletion and 100 μg of anti-NK1.1 mAb every 5 days to maintain depletion (14).

FACS analysis

Purified cells were stained with the following Abs to mouse Ags: NK1.1, CD3, CD4, and annexin-V (all obtained from BD Biosciences). The cell surface receptor for IL-21 was detected with the use of biotinylated IL-21 (R&D Systems) as described previously (28). All samples were analyzed on a FACSaria or FACCsCalibur using Diva or CellQuest software (BD Biosciences).

NK cell cytotoxicity assay

NK cell-mediated cytotoxicity was assayed using a standard 51Cr release assay (14).

Cytokine ELISA and ELISPOT

Levels of IL-21 in culture supernatants of AChR-reactive T cells during EAMG were quantified by ELISA (mouse rIL-21 and goat anti-IL-21; all obtained from Capralogics). The IFN-γ ELISPOT assay was described previously (14).

Lymphocyte labeling with CFSE

Spleen cells were incubated with CFSE at a concentration of 0.5 μM at 37°C for 30 min. Cells were washed and seeded in plates at a concentration of 4 × 10^5 cells/well. Cells were incubated with or without LPS (1 μg/ml). After 72 h of incubation, the cells were washed, and dilution of CFSE among NK1.1−CD3− cells was analyzed by FACS.

Results

NK cell degeneration during the progression of autoimmunity

To understand the mechanism underlying the degeneration of NK cells in human autoimmune disease, we adopted EAMG in C57BL/6 (B6) mice, an animal model for the classic T cell-driven, B cell-mediated autoimmune disease MG (14). To produce the muscle weakness characteristic of MG in B6 mice, repeated immunization with AChR in CFA (or IFA) is required (29). Most mice develop clinical overt muscle weakness after the second immunization, and disease becomes progressive in most animals. The advantage of this model is that the target autoantigen AChR is well defined (26) and that the course of EAMG mirrors both the initiation and progression phases of the human disease. The inception of MG and other autoimmune disorders is virtually impossible to monitor in most humans, because they represent clinically silent events occurring many years before clinical presentation of the disease in most patients. Additionally, multiple immunizations serve as a surrogate for environmental triggering in humans and offer sustained NK cell stimuli.

To understand the mechanism underlying the degeneration of NK cells during the progression of EAMG, we initiated our investigation by immune-phenotyping and functional characterization of NK cells during the course of EAMG. In mice immunized with AChR/CFA, splenic NK cells appeared to undergo rapid proliferation from day 1 to 3 postimmunization (p.i.), as evidenced by dilution of CFSE on gated NK cells (Fig. 1a), followed by a numerical decline after day 7 p.i. By day 75 p.i., the percentage and absolute number of NK cells (NK1.1−CD3+) was approximately one-fourth of that in the induction phase of EAMG (Fig. 1, b and d). The dynamic changes in the NK cell compartment during the initiation of EAMG appeared to be the result of NK cell proliferation in situ rather than recruitment, because modest NK cell expansion was also observed in lymph nodes, peripheral blood, and thymus (data not shown). In contrast, there was a moderate increase in the percentage of NK cell in mice injected with CFA at the same frequency, which was maintained throughout the 100-day observation period (Fig. 1, b and c). NK cells from mice treated with AChR/CFA rapidly acquired the ability to lyse YAC-1 target cells (Fig. 1e) and to spontaneously produce IFN-γ (Fig. 1f) at days 3 and 7 p.i. However, from day 15 p.i., NK cell activity started to decline. This deficit in NK cell function was also observed when purified NK cells were activated with LPS (1 μg/ml) (numbers of IFN-γ spot-forming colonies in EAMG mice were 47 ± 11 vs 13 ± 4 on day 3 and 75 p.i.). In contrast, mice immunized with CFA alone maintained NK cell functions at a magnitude similar to that preceding their initial priming (Fig. 1, e and f).

We also examined the kinetics of T cell and autoantibody responses to AChR and its dominant epitope α146–162 during the course of EAMG. Vigorous T cell proliferation and anti-AChR-producing B cells in response to AChR and α146–162 emerged around day 6 and 8 p.i., respectively (Fig. 1, g and h, and data not shown), just before the decline in NK cell functions. We observed a similar pattern of NK cell degeneration during progression of the autoimmune response to myelin Ags, including myelin basic protein and myelin oligodendrocyte glycoprotein, whereas the number and functions of NK cells were maintained in mice immunized with CFA and OVA (F.-U. Shi, unpublished observation). These results suggest that 1) NK cells undergo significant degeneration during the transition from EAMG initiation to disease progression; 2) the observed decline in NK cell functions is not caused by the lack of continuous stimuli; and 3) this transition in NK cell function is associated with the emergence of autoreactive T and B cell responses.
Maintenance of NK cell function in mice devoid of CD4+ T cells

The data so far suggest that the interaction of NK cells and autoreactive T and B cells at different stages of autoimmune disease might be very different in nature. RAG1−/− mice have intact cellular components of innate immunity but do not posses T and B cells (21). Immunization of RAG1−/− mice with AChR/CFA activates the innate immune system without mounting autoreactive T and B cell responses. Therefore, this strain provides an opportunity to determine whether NK cells become degenerative in the absence of autoreactive T and B cells. Immunization of RAG1−/− mice with AChR and CFA resulted in increased numbers and percentages of NK cells, as well as numbers of IFN-γ-producing cells, as compared with similarly immunized wild-type mice (due to lack of a T and B cell compartment). These levels remained constant from day 1 to 61, and were comparable to levels in CFA-injected RAG1−/− mice (Table I, group 1 and 2). In contrast, the functional characterization of NK cells in μMT mice (deficient in B cells) revealed a decline in NK cell function similar to that in wild-type mice (p < 0.05 for comparison of numbers of NK cells and numbers of IFN-γ spot-forming cells at day 1 and 61 p.i.; Table I, group 3 and 4). Similar results were obtained in AChR/CFA-primed NKT cell-deficient CD1d1 knockout mice (group 9 and 10). These results suggest that the degeneration of NK cells is mediated by T cells and rule out significant contributions of B cells and NKT cells.

Having determined that NK cell functions are preserved in mice devoid of T cells, we asked whether CD4+ or CD8+ T cells mediate NK cell degeneration in EAMG. For this purpose, we examined NK cell prevalence and functions in AChR/CFA-primed mice with disrupted genes encoding for CD4 or CD8α. Compared with wild-type mice, AChR/CFA-primed CD8−/− mice had fewer NK cells, reduced capacity to kill YAC-1 targets, and decreased production of IFN-γ (Table I, group 7 and 8). Remarkably, neither the prevalence of NK cells, nor the killing of YAC-1 targets and production of IFN-γ were significantly altered in the AChR/CFA-primed CD4−/− mice (Table I, group 5 and 6). These data strongly suggest that CD4+ T cells are the primary mediators of NK cell degeneration.

IL-21R+ NK cells are prone to cell death

NK cells in environments of ongoing autoimmunity may be confronted with excess quantities of cytokines derived from autoreactive T cells. The shared common γ-chain-binding cytokines that mediate NK cell expansion and survival include IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21 (23, 24, 28, 30–33). IL-21 is the cytokine most likely to confer NK cell degeneration because 1) IL-21 is produced only by CD4+ T cells, but not by CD8+ T cells or other types of lymphocytes; and 2) the effects of IL-21 on NK cells depend on their functional status (24, 28).

In an attempt to link IL-21 with NK cell degeneration during the course of EAMG, we quantified the levels of IL-21 production by AChR-reactive T cells by ELISA. IL-21 became detectable at ~7 days p.i. and was maintained at a relatively high level during the progression of EAMG (Fig. 2a). Next, we determined whether the IL-21R could mark distinct populations of NK cells for survival or death. Using FACS analysis, we probed the surface characteristics of cell death of NK cells at different stages of EAMG. We found that annexin-V expression was greater on gated IL-21R+ NK cells than on IL-21R− NK cells (6.3 vs 11.9%; p < 0.05) at days 3 to 15 after AChR/CFA immunization (Fig. 2b, top two panels). Annexin-V levels were further increased on IL-21R+ NK cells examined on day 61 p.i. (Fig. 2b, bottom panel). The IL-21R+ NK cells constituted ~22% of total NK cells at day 15 of EAMG (Fig. 2c). Thus, IL-21R+ NK cells are more susceptible to apoptosis than IL-21R− NK cells.

IL-21 produced by autoreactive CD4+ T cells mediates NK cell degeneration

To determine whether cytokines produced by autoreactive CD4+ T cells are indeed responsible for NK cell degeneration, we performed NKT cell coculture experiments. NK cells cultured with the supernatant of AChR-reactive CD4+ cells, or with CD4+ cells themselves (with or without Transwell), produced less IFN-γ than...
controls (Fig. 3a), and the number of NK cells was slightly (NS) decreased as compared with controls (data not shown). These results indicated that AChR-reactive CD4⁺ T cell-derived factors are responsible for functional decay in the coculture.

Because IL-21R⁻/⁻ NK cells are short lived, we investigated whether IL-21 produced by AChR-reactive CD4⁺ T cells mediates NK cell degeneration. No blocking Abs to IL-21 are available, which prevented us from addressing this issue in NKT cell coculture experiments. Instead, we reasoned that, if IL-21 indeed mediates NK cell degeneration, IL-21R⁻/⁻ NK cells would not undergo such degeneration during the progression of EAMG. To assess this possibility, we immunized wild-type and IL-21R⁻/⁻ mice (24) with AChR/CFA. Compared with wild-type mice, IL-21R deficiency had no significant alterations in T cell proliferative responses to AChr and to its dominant epitope α146–162 (data not shown). Furthermore, NK cell numbers (Fig. 3b), cytotoxicity (Fig. 3c), and IFN-γ production (Fig. 3d) remained unaltered. Thus, we have identified IL-21 as a primary mediator of NK cell degeneration in EAMG.

To determine the contributions of other cytokines that may also influence NK cell survival and function during the progression of autoimmunity to AChR, we examined NK cell prevalence and functions in AChR/CFA-primed IL-12⁻/⁻, IL15⁻/⁻ (23), and IL-18 mice (24) with AChR/CFA. Compared with wild-type mice, IL-12 deficiency had no significant alterations in T cell proliferative responses to AChr and to its dominant epitope α146–162 (data not shown). These results indicated that AChR-reactive CD4⁺ T cells undergo such degeneration during the progression of EAMG. To assess this possibility, we immunized wild-type and IL-21R⁻/⁻ mice (24) with AChR/CFA. Compared with wild-type mice, IL-21R deficiency had no significant alterations in T cell proliferative responses to AChr and to its dominant epitope α146–162 (data not shown). Furthermore, NK cell numbers (Fig. 3b), cytotoxicity (Fig. 3c), and IFN-γ production (Fig. 3d) remained unaltered. Thus, we have identified IL-21 as a primary mediator of NK cell degeneration in EAMG.

AChR primed-IL-21R⁻/⁻ mice develop exacerbated autoimmune

To understand whether NK cell degeneration is an epiphenomenon that develops secondary to autoreactive T cell activation, or NK cell degeneration can impact the progression of autoimmunity, we immunized control mice and IL-21R⁻/⁻ mice with AChR/CFA and evaluated the development of EAMG. The median day of onset of muscular weakness of MG in control mice and in IL-21R⁻/⁻ mice. In agreement with previous studies (13), AChR/primed IL-12⁻/⁻ and IL-18⁻/⁻ mice displayed various degrees of functional NK cell defects compared with control animals (Table II). However, the functional decline continued in these mice throughout the 90-day observation period (Table II). Furthermore, the numerical decrease in NK cells of AChR/CFA-immunized IL-12⁻/⁻ and IL-18⁻/⁻ mice resembled that in wild-type mice (Table II). In a previous report, it was shown that IL-15⁻/⁻ mice have markedly reduced NK cell numbers (23). We found that NK cell numbers could not be rescued by AChR/CFA immunization. Although NK cell numbers did not significantly alter during the 90-day observation period, production of IFN-γ by the remaining NK cells declined (Table II). Therefore, the contribution of IL-12, IL-15, and IL-18 to NK cell degeneration in autoimmune disease, if any, is minimal.

![FIGURE 3](http://www.jimmunol.org/Downloaded_from)

**FIGURE 3.** IL-21 mediates NK cell degeneration. Wild-type (control) mice and IL-21R⁻/⁻ mice were immunized with AChR/CFA at 3 monthly intervals to induce EAMG. We enriched CD4⁺ T cells from the pooled splenocytes of AChR/CFA-primed mice (day 15 p.i.) and cultured these cells with irradiated autologous splenocytes serving as APC in the presence of AChr (10 μg/ml). AChR-reactive CD4⁺ clones were selected using a “split-well” assay (27). NK cells from AChR/CFA-primed mice (day 3 p.i.) were purified and then cultured for 24 h with supernatants from AChR-reactive CD4⁺ T cell cultures or with AChR-reactive CD4⁺ T cells themselves. a, NK cells from wild-type or IL-21R⁻/⁻ spleen cells incubated for 48 h with culture medium (control) or supernatant from AChR-reactive CD4⁺ T cells cultures or CD4⁺ T cell cultures from wild-type mice. Mean numbers (±SD) of IFN-γ spot-forming NK cells were measured by ELISPOT assay. In some experiments, NK cells and CD4⁺ T cells were cultured in Transwells. After 48-h culture, CD4⁺ cells were removed from culture by a mAb. b, Percentages of NK cells among splenocytes at days 3 and 75 p.i. from wild-type mice and IL-21R⁻/⁻ mice were determined by FACS. c, NK cell-mediated cytotoxicity of splenocytes from wild-type mice and IL-21R⁻/⁻ mice against ⁵¹Cr-labeled YAC-1 target cells at an E:T ratio of 100:1. d, Mean numbers (±SD) of IFN-γ spot-forming NK cells were measured by ELISPOT assay. NK cells from mice indicated in b were enriched from the spleen, sorted by flow cytometry, and cultured for 48 h without Ag stimulation. Data shown represent one of two experiments with n = 4/group. Results were similar for cells from draining lymph nodes (data not shown). Statistical comparisons were performed between AChR/CFA and CFA groups, or between time points within a group. *p < 0.05.
Comparisons were made between day 1 p.i. and day 61 p.i. within the same group of mice. In agreement with a previous report (31), we found that levels of AChR-reactive IgG1 were decreased in IL-21R–/– mice (data not shown). Therefore, the competent NK cells in IL-21R–/– mice promoted both NK and NKT cells, we cross-bred the CD1d double-mutant mice reversed these effects (data not shown). NK cell degeneration may signify the transition from innate immune responses to AChR. These findings suggest that NK cell degeneration functions to avoid excessive T cell-mediated autoimmunity.

Discussion

The paradigm that innate immunity plays an instructive role in the acquired immune response is well established (3, 6). We now provide evidence that adaptive autoimmune T cell responses can affect the prevalence and function of NK cells, a major cellular player of innate immunity. Our results provide an answer for the long-standing question of how and why NK cells degenerate in the course of human autoimmune disease. Furthermore, the present results also provide a likely mechanistic explanation for earlier findings indicating that depletion of NK cells after T cell activation no longer significantly affects the incidence and severity of experimental autoimmune diseases (13, 14).

A time-honored debate is whether NK cell defects are a primary feature of autoimmune disease or secondary either to pathogenesis or therapeutic intervention (6, 7, 17). The association between decreased NK cell function and autoimmunity has fueled an attractive hypothesis that autoimmunity results from a lack of suppressive influence of NK cells (19). Our results do not support this notion. Instead, NK cells promoted the genesis of autoreactive Th1 cells and disease during the initiation of autoimmunity (13, 14). As portrayed in this study, once autoreactive T cells came into play, NK cells were controlled by T cell-derived cytokines that led to a functional deficiency and partial deletion of NK cells. Thus, this NK cell degeneration may signify the transition from innate immune triggering to the emergence of adaptive (autoreactive) T cells. The stage of an autoimmune response therefore determines the nature of NKT cell interactions.

The question arises whether NK cell degeneration is just an epiphenomenon that occurs secondary to autoreactive T cell activation, or whether NK cell degeneration has any impact on the progression of autoimmunity. Our results revealed that IL-21R–/– mice, with competent NK cells, developed exacerbated autoimmunity.

Table II. NK cell functions in autoantigen-primed mice devoid of IL-12, IL-15, and IL-18

<table>
<thead>
<tr>
<th>Groups (mice)</th>
<th>No. of Mice</th>
<th>Treatment</th>
<th>Percentage of No. NK Cells (%)</th>
<th>No. NK Cells (×10⁶) Day 1 p.i.</th>
<th>Percentage of No. NK Cells (%)</th>
<th>No. NK Cells (×10⁶) Day 61 p.i.</th>
<th>IFN-γ-Forming Cells/10⁶ NK Cells Day 1 p.i.</th>
<th>IFN-γ-Forming Cells/10⁶ NK Cells Day 61 p.i.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. C57BL/6</td>
<td>4</td>
<td>AChR/CFA</td>
<td>2.3 ± 0.3</td>
<td>19.8 ± 4.3</td>
<td>0.6 ± 0.6**</td>
<td>7.6 ± 1.0**</td>
<td>29.6 ± 3.1</td>
<td>11.1 ± 4.8*</td>
</tr>
<tr>
<td>2. IL-12−/−</td>
<td>7</td>
<td>AChR/CFA</td>
<td>1.9 ± 0.4</td>
<td>17.0 ± 1.2</td>
<td>0.9 ± 0.2</td>
<td>8.1 ± 2.5</td>
<td>17.8 ± 3.5</td>
<td>6.2 ± 2.1</td>
</tr>
<tr>
<td>3. C57BL/6</td>
<td>6</td>
<td>AChR/CFA</td>
<td>2.0 ± 0.4</td>
<td>18.4 ± 2.3</td>
<td>0.5 ± 0.1**</td>
<td>5.6 ± 1.5</td>
<td>33.8 ± 5.2</td>
<td>14.2 ± 2.6*</td>
</tr>
<tr>
<td>4. IL-15−/−</td>
<td>8</td>
<td>AChR/CFA</td>
<td>0.43 ± 0.1</td>
<td>0.2 ± 0.3</td>
<td>0.41 ± 0.2</td>
<td>0.5 ± 0.2</td>
<td>21.1 ± 11.3</td>
<td>10.4 ± 6.5*</td>
</tr>
<tr>
<td>5. C57BL/6</td>
<td>4</td>
<td>AChR/CFA</td>
<td>2.7 ± 0.3</td>
<td>21.2 ± 5.2</td>
<td>1.1 ± 0.6*</td>
<td>10.6 ± 4.1*</td>
<td>37.6 ± 5.6</td>
<td>17.2 ± 5.1*</td>
</tr>
<tr>
<td>6. IL-18−/−</td>
<td>6</td>
<td>AChR/CFA</td>
<td>2.2 ± 0.3</td>
<td>19.5 ± 2.6</td>
<td>0.9 ± 0.5*</td>
<td>9.4 ± 2.5*</td>
<td>16.1 ± 8.1</td>
<td>9.4 ± 5.2*</td>
</tr>
</tbody>
</table>

* Groups of mice were immunized with AChR/CFA at 3 monthly intervals. Percentages of NK cells among splenocytes at day 1 and 61 after primary immunization were analyzed by FACS. Mean numbers (±SD) of IFN-γ, spot-forming NK cells were measured by ELISPOT. Results represent one of two experiments with n = 4/group. Comparisons were made between day 1 p.i. and day 61 p.i. within the same group of mice. *, p < 0.05; **, p < 0.01.

Table III. NK cells are responsible for the exacerbated autoimmunity in IL-21R−/− mice

<table>
<thead>
<tr>
<th>Groups (mice)</th>
<th>No. of Mice</th>
<th>Treatment</th>
<th>Average Cumulative Disease Score</th>
<th>IFN-γ (pg/ml)</th>
<th>IgG1 (OD405)</th>
<th>IgG2a (OD405)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. B6</td>
<td>11</td>
<td>None</td>
<td>18.7 ± 6.3</td>
<td>247.9 ± 18.8</td>
<td>1.9 ± 0.3</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>2. IL-21R−/−</td>
<td>14</td>
<td>None</td>
<td>28.6 ± 12.1*</td>
<td>439.9 ± 21.0*</td>
<td>2.6 ± 0.2*</td>
<td>0.3 ± 0.5*</td>
</tr>
<tr>
<td>3. IL-21R−/−</td>
<td>8</td>
<td>Mouse IgG2a</td>
<td>22.5 ± 5.5</td>
<td>475.8 ± 21.3</td>
<td>2.7 ± 0.3</td>
<td>1.0 ± 0.3</td>
</tr>
<tr>
<td>4. IL-21R−/−</td>
<td>8</td>
<td>Anti-NK1.1 (day-2)</td>
<td>14.2 ± 3.7*</td>
<td>294.3 ± 12.0*</td>
<td>1.5 ± 0.3*</td>
<td>0.5 ± 0.1*</td>
</tr>
<tr>
<td>5. IL-21R−/−</td>
<td>8</td>
<td>Mouse IgG2a</td>
<td>24.3 ± 6.2</td>
<td>445.0 ± 23.5</td>
<td>2.1 ± 0.2</td>
<td>0.8 ± 0.2</td>
</tr>
<tr>
<td>6. IL-21R−/−</td>
<td>8</td>
<td>Anti-NK1.1 (day 15)</td>
<td>15.7 ± 5.0*</td>
<td>235.2 ± 14.4*</td>
<td>1.6 ± 0.3*</td>
<td>0.3 ± 0.1**</td>
</tr>
</tbody>
</table>

* Groups of mice were immunized with AChR/CFA at 2 monthly intervals. Groups 3–6 received control Abs or anti-NK1.1 mAb at different time points as indicated. AChR-induced production of IFN-γ and IgG1, IgG2a were measured by ELISA (OD405) and presented as mean numbers ± SD. Comparisons were made between corresponding groups. *, p < 0.05; **, p < 0.01.
autoimmunity against AChR. Depletion of NK cells in IL-21R−/− mice abolished these effects. Thus, NK cell degeneration would have a profound impact on progression of autoimmunity. In other words, if autoreactive T cells do not turn off NK cells, as in the case of IL-21R−/− mice, autoimmunity can become excessive.

In pathophysiological circumstances, NK cells and T cells can be colocalized in several peripheral lymphoid compartments including lymph nodes (34, 35). This anatomical proximity enhances the likelihood of direct/indirect interactions between NK cells and T cells. Accumulating evidence suggests that the cross-talk between NK cells and T cells can be mediated by APCs (e.g., dendritic cells) or via costimulatory molecules and cytokines. Dendritic cells may carry NK cell-derived helper signals to direct differentiation of Th1 cells (36, 37).

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Disclosures

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References


